

Kit 6 comprises optical fiber 12, capillary tube 14, and stopper 16.

Fiber 12 is an elongate substantially cylindrical optically transparent body adapted to propagate along its length by multiple total internal reflections, optical radiation entering an end of the fiber within an established solid angle substantially rotationally symmetric about the fiber's axis. As is well known in the art of fiber optics, the maximum acceptance angle, with regard to the fiber axis, B, for the radiation entering the fiber and so propagated within it, is established by the refractive indices of the fiber and the surrounding medium. For radiation initially propagating through a medium of refractive index n_0 , incident upon a fiber of refractive index n_1 otherwise surrounded by a material of refractive index n_2 , the maximum acceptance angle may be found from the equation

$$N.A. = n_0 \sin B = (n_1^2 - n_2^2)^{1/2} \quad (1)$$

where N.A. is the so-called numerical aperture of the fiber. By way of example, but not limitation, fiber 12 may be any of a number of optically transparent materials, such as glass, quartz, polypropylene, polyolefin, nylon, or the like, chosen to have an index of refraction greater than that of the fluid sample being assayed. The latter typically is an aqueous solution having an index of refraction near 1.33 or a serum sample having an index of refraction near 1.35. The fiber is further chosen of a material that is relatively insoluble and non-reactive with the fluid sample. It has been found that 200 microns is a satisfactory fiber diameter, although other fiber diameters may be used. For most assays, a fiber 25 mm in length appears adequate; however, it will be understood that the length of the fiber can be accommodated to the assay to be undertaken. Sensitivity in fact improves with increase in fiber length until signal attenuation over the fiber length exceeds $1/e$.

As will be described in detail hereinafter, fiber 12 is provided with a surface coating including means for attaching selected moieties of an antigen-antibody complex. As herein used, the term "antigen-antibody complex" includes complexes not only of complete antibodies and antigens, but complexes incorporating immunologically reactive fragments of either or both.

Capillary tube 14 is preferably an optically transparent tube, its material of construction also being chosen to be relatively insoluble and nonreactive with the fluid sample being assayed. Thus, capillary tube 14 is preferably fabricated from such materials as glass, quartz, polypropylene, polyolefin, or the like. In a preferred embodiment, capillary tube 14 is of right circular cylindrical bore, having an inside diameter a slightly larger than the diameter of fiber 12 (e.g., for a fiber diameter of 200 microns, capillary tube 14 may have an inside diameter of about 240 microns). However, as will be described, the invention may be practiced with capillary tubes of considerably greater internal diameter than the fiber.

Stopper 16 is configured and dimensioned to fit within an end of capillary tube 14 and support an end portion 18 of fiber 12 substantially coaxially and in spaced-apart relation within the capillary tube. Additionally, stopper 16 provides a hard locating surface for positioning kit 6 in a fluorimeter as will be described hereinafter. To these ends, stopper 16 is preferably provided with a flange 20 having an overall diameter on the order of the outside diameter of capillary tube 14 and a centrally disposed ferrule-like extension 21 coaxial with a central bore 22. Bore 22 penetrates throughout stop-

per 16, and is dimensioned to secure end portion 18 of fiber 12. In a preferred embodiment, stopper 16 is molded in place about fiber 12, the stopper being preferably fabricated of a low refractive index material, such as siloxane. Fiber 12 passes through and is supported by stopper 16 so as to expose substantially all of the fiber but end portion 18 to the interior of capillary tube 14, leaving end face 24 of end portion 18 unobscured and coterminous with the extremity of bore 22 external to the capillary tube.

End face 24 is preferably planar and disposed normally to the axis of fiber 12. Preferably, end face 24 is also highly transparent and free of blemishes which would tend to scatter light incident upon the end face. To this end, end face 24 may be optically polished, although it has been found that fused quartz fiber may be cleaved to provide an adequate optical surface.

Optionally, the end face 26 of the fiber distal from end face 24 is also polished flat or cleaved and further provided with a mirror coating 28 (or a separate mirror) disposed substantially normal to the fiber axis, thereby causing radiation trapped in the fiber to double-pass the fiber.

The overall dimensions of fiber 12, capillary tube 14, and stopper 16 preferably are chosen to insure that end face 26 of the fiber is within the capillary tube.

It will be realized that kit 10 as thus described is essentially the same as that taught in U.S. Pat. No. 4,447,546. Importantly however in the present invention, tube 14 is provided with port 30 in fluid communication with the interior of tube 14 distal the open end of the tube. In a preferred embodiment, port 30 is in the form of a tubular conduit 32 provided with a septum 34. Tubular conduit 32 is located proximate stopper 16 and extending radially from tube 14. Septum 34 is adapted, as well known in the art, to be penetrated by a hypodermic needle (not shown), and the interior of conduit 32 is dimensioned to accommodate the needle intended for use with the system. It will be understood that port 30 might equally well be the appropriate coupling for attachment to a Luer lock-equipped syringe, or some equivalent apparatus.

Prior to being assembled into kit 10, fiber 12 is provided a coating, as will be described, activating a region 36 of the cylindrical surface of the fiber for the assay to be performed. In a preferred embodiment, the activated region 36 is restricted to a predetermined length of fiber 12 by a chemically and optically inert coating 38 of, for instance, low optical index silicone, extending over both ends of the fiber. It will be understood, however, that the dimensions of activated region 36 may be controlled by other means (e.g., by masking the fiber during coating), or, alternatively, the entire length of fiber 12 might be activated and the length of the fiber disposed within the capillary tube to be carefully controlled.

Turning now to FIG. 3, there may be seen a highly stylized representation of a longitudinal crosssectional portion of kit 2 within activated region 36 of fiber 12, filled with a sample 43 to be assayed. The surface of fiber 12 within region 34 is provided with a plurality of coupling sites 44, to a number of which are bound a moiety 46 of the antibody-antigen complex. As used herein, the phrase "moiety of an antibody-antigen complex" refers to an immunologically reactive portion of such a complex, and includes haptens as well as complete antigens and antigen reactive antibody fragments [Fab] as well as complete antibodies. Coupling sites 44